Application Serial No. 10/629,895 Amendment and Response to Restriction

Requirement dated 16 December 2010

Reply to Office Action dated 16 November 2010

AMENDMENTS TO THE SPECIFICATION

Please insert the following new heading and new paragraph before the FIELD OF THE

INVENTION on page 1 of the application as filed.

SEQUENCE SUBMISSION

The present application includes a Sequence Listing filed in electronic format. The Sequence

Listing is entitled 1954-413_Sequence_Listing.txt, was created on 16 November 2010 and is 2 kb in

size. The information in the electronic format of the Sequence Listing is part of the present

application and is incorporated herein by reference in its entirety.

Please substitute the following replacement paragraph for paragraph [00039] of the

application as filed.

[00039] VA1-miRNAs and VA1-siRNAs can contain 1) 21-nt sense, 2) 4, 8, or 9 base loop for siRNA or miRNA (mir-30 or let-7a-3), and 3) 21-nt antisense strand complementary to a HIV-1

rev target (total ~50-nt). To make inactive mutant mi- or si-RNA variants, 4-nt in the middle of the

stem sequences were mutated to be non-complementary to the target. BstEII restriction fragments

containing mi- or si-RNA sequences are prepared from synthetic oligonucleotides, which share 12

bases of complementary sequence at their 3' end. These were annealed and the primer ends

extended using Taq polymerase and several rounds of PCR. The wt and mt mi- or si-RNA

sequences are cloned into the BstEII site of pVA1 (Fig.1A). The sequences of the loops are: 4 base loop: TTAA [SEQ ID NO: 4], 8 base loop: GAAGCTTG [SEQ ID NO: 5] (underlined: HindIII site),

and 9 base loop: TTCAAGAGA [SEQ ID NO: 6]. As shown in Fig. 1B, the DNA sequence (SEQ

ID NO:7) encoding a short hairpin RNA cloned into the BstEII site of pVA1 comprises a 21 nt sense

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strand, a 8 nt hairpin loop and a 21 nt antisense strand complementary to a HIV-1 rev target. The 8 nt hairpin loop shown in Fig. 1B is SEQ ID NO:8,